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(54) Title: VACCINE AGAINST SEXUALLY TRANSMITTED DISEASES

(57) Abstract

A method of administering a vaccine to females to prevent or treat infections associated with pathogens which cause sexually transmitted diseases is described. The vaccine comprises one or more antigens for the prevention or treatment of sexually transmitted diseases, for example an HSV glycoprotein D or an immunological fragment thereof, and an adjuvant, especially a TH-1 inducing adjuvant. The use of the vaccine components for the formulation of a vaccine composition for the prevention or treatment of sexually transmitted diseases in female subjects is also described.

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VACCINE AGAINST SEXUALLY TRANSMITTED DISEASES

The present invention relates to one or more antigens for the prevention or treatment of sexually transmitted diseases and the use thereof in the formulation of a vaccine, for administration to female human subjects, for the prevention or treatment of infections associated with pathogens which cause sexually transmitted diseases. The invention also relates to a method of administering the vaccine to females to prevent or treat infections associated with pathogens which cause sexually transmitted diseases.

Pathogens which cause sexually transmitted diseases (STDs) are known and there is an urgent need for effective vaccines to treat or prevent such conditions.

Sometimes sexually transmitted diseases are caused by one or more pathogens. Combination vaccines, able to prevent and/or treat, one or more STDs are therefore also required.

It has been found that certain vaccine formulations are surprisingly efficiacious in preventing or treating STDs in female human subjects who are susceptible to or suffering from such STDs.

The present invention provides a method of treating a female human subject suffering from or susceptible to one or more sexually transmitted diseases (STDs), which method comprises administering to a female subject in need thereof an effective amount of a vaccine formulation comprising one or more antigens derived from or associated with a STD-causing pathogen and an adjuvant.

Preferably the adjuvant is a TH-1 inducing adjuvant.

In a related aspect the invention provides the use of one or more antigens derived from or associated with a STD-causing pathogen and an adjuvant, especially a TH-

1 inducing adjuvant, in the preparation of a vaccine for administration to a human female subject for the prevention and/or treatment of one or more STDs.

Examples of antigens derived from or associated with a STD-causing pathogen include those derived from or associated with herpes viruses (HSV-1 and HSV-2), human papillomaviruses (HPV- all types), Chlamydia trachomatis, Neiserria gonnorhea, Treponema pallidum (syphilis) and Haemophilus ducreyi (chancroid).

Other sources of antigens including recombinant bacteria, recombinant viruses, fusion proteins, peptides and mimotopes may also be used.

The above list is not exhaustive and other pathogens are well known to medical practitioners and others skilled in the art and are listed in standard textbooks.

Suitable adjuvants for use in the invention include those well known in the art of vaccine formulation. By 'TH-1 inducing adjuvant' is meant an adjuvant which is a preferential stimulator of TH1 cell response.

A recognised signal that a TH1 response has been stimulated is the enhanced production of TH1-type cytokines *eg*. IFN-γ and IL-2. IFN-γ secretion is associated with protective responses against intracellular pathogens, including parasites, bacteria and viruses. Activation of leucocytes by IFN-γ enhances killing of intracellular pathogens and increases expression of Fc receptors. Direct cytotoxicity may also occur, especially in synergism with lymphotoxin (another product of TH1 cells). IFN-γ is also both an inducer and a product of NK cells, which are major innate effectors of protection. TH1 type responses, either through IFN-γ or other mechanisms, provide preferential help for murine IgG2a immunoglobulin isotypes.

In contrast, TH-2 type responses are associated with humoral mechanisms and the secretion of IL-4, IL-5, IL-6, IL-10 and tumour necrosis factor-beta.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application Nos. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred 'small particle' form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP O 689 454B1 (SmithKline Beecham Biologicals SA).

In such 'small particle' 3-DMPL the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (as described in European Patent number 0 689 454).

Another preferred adjuvant which may be used in the present invention comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed (as QS21) in US patent No. 5,057,540 and is available from Aquilla Pharmaceuticals.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen. Thus vaccine compositions which form part of the present invention may include a combination of QS21 and cholesterol.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt. Other mineral salts may also be used as a carrier such as salts of calcium, iron or zinc. Other carriers include polyphosphazene, liposomes and ISCOMS.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline. A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. Additionally the oil in water emulsion may contain span 85 and/or lecithin.

Typically for human administration QS21 and 3D MPL will be present in a vaccine in the range of 1μg - 500μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal or less than 1 as this provides amore stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

In a preferred aspect aluminium hydroxide (alum) or aluminium phosphate will be included in the vaccine composition which is used or manufactured according to the invention.

In a particularly preferred aspect the antigens in the vaccine composition used or manufactured according to the invention are combined with 3D-MPL and alum.

Vaccines employed in the present invention may, if desired, comprise adjuvant molecules of general formula (I):

$HO(CH_2CH_2O)_n$-A-R

wherein, n is 1-50, A is a bond or -C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12^{th} ed: entry 7717).

HSV-2 is the primary etiological agent of herpes genitalis. HSV-1 is the causative agent of herpes labialis. Together, these viruses are characterised by their ability to induce both acute diseases and to establish a latent infection, primarily in neuronal ganglia cells.

WO 92/16231 provides further background information about genital herpes and describes a vaccine which can be used to treat people susceptible to HSV infections comprising HSV glycoprotein D or an immunological fragment thereof in conjunction with 3-O-deacylated monophosphoryl lipid A and a suitable carrier.

The specification of WO 92/16231 provides details of glycoprotein D, immunological fragments thereof, and 3-DMPL and methods for obtaining it. The specification describes some promising tests of a candidate vaccine in animal models but no data in humans are given.

In a preferred aspect the method or use according to the invention relates to the prevention or treatment of infections associated with genital herpes, in particular HSV-2 infections.

The vaccine which may be used in the present invention comprises glycoprotein D or an immunological fragment thereof which is typically from HSV-2.

Glycoprotein D is located on the viral membrane, and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al; J of Virol 1980 35 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60 kD. Of all the HSV envelope glycoproteins this is probably the best characterised (Cohen et al J. Virology 60 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be able to elicit neutralising antibodies in vivo (Eing et al J. Med. Virology 127: 59-65). However, latent HSV-2 virus can still be reactivated and induce recurrence of the disease despite the presence of high neutralising antibodies titre in the patients sera.

As described in WO 92/ 16231, a preferred embodiment thereof is a truncated HSV-2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 of the naturally occurring glycoprotein with the addition of aparagine and glutamine at the C-terminal end of the truncated protein devoid of its membrane

anchor region. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The production of such a protein in Chinese Hamster Ovary cells has been described in EP – B- 139 417.

The mature truncate preferably used in the vaccine formulation within the scope of the invention may be designated recombinant gD2t (rgD2t) or simply (as hereinbelow) gD2t.

The HSV antigen may be chemically or otherwise conjugated to a particulate carrier as described in WO 92/16231.

In one preferred aspect the vaccine for use in the invention comprises gD2t, 3-DMPL (especially small particle 3-DMPL) and aluminium hydroxide (alum).

Papillomaviruses are small DNA tumour viruses, which are highly species specific. As yet, over 70 individual human papillomavirus (HPV) genotypes have been described. HPVs are generally specific either for the skin (eg HPV-1 and -2) or mucosal surfaces (eg HPV-6 and -11) and usually cause benign tumours (warts) that persist for several months or years. Such benign tumours may be distressing for the individulas concerned but tend not to be life threatening, with a few exceptions.

Some HPVs are also associated with cancers. The strongest positive association between an HPV and human cancer is that which exists between HPV-16 and HPV-18 and cervical carcinoma. Cervical cancer is the most common malignancy in developing countries, with about 500,000 new cases occuring in the world each year. It is now technically feasible to actively combat primary HPV-16 infections, and even established HPV-16-containing cancers, using vaccines. For a review on the prospects for prophylactic and therapeutic vaccination against HPV-16 see Cason J., Clin. Immunother. 1994; 1(4) 293-306 and Hagenesee M.E., Infections in Medicine 1997 14(7) 555-556,559-564. Preferably a vaccine composition according to the invention comprises the major capsid protein, the L1 protein.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well characterised bovine papillomavirus type 1 (BPV1).

Although minor variations do occur, all HPVs genomes described have at least seven early genes, E1 to E7 and two late genes L1 and L2. In addition, an upstream regulatory region harbors the regulatory sequences which appears to control most transcriptional events of the HPV genome.

E1 and E2 genes are involved in viral replication and transcriptional control, respectively and tend to be disrupted by viral integration. E6 and E7, and recent evidence implicate also E5 are involved in viral transformation.

In the HPVs involved in cervical carcinoma such as HPV 16 and 18, the oncogenic process starts after integration of viral DNA. The integration results in the inactivation of genes coding for the capsid proteins L1 and L2 and in installing continuously over expression of the two early proteins E6 and E7 that will lead to gradually loss of the normal cellular differentiation and the development of the carcinoma.

Carcinoma of the cervix is common in women and develops through a pre-cancerous intermediate stage to the invasive carcinoma which frequently leads to death. The intermediate stages of the disease is known as cervical intraepithelial neoplasia and is graded I to III in terms of increasing severity.

Clinically, HPV infection of the female anogenital tract manifests as cervical flat condylomas, the hallmark of which is the koilocytosis affecting predominantly the superficial and intermediate cells of the cervical squamous epithelium.

Koilocytes which are the consequence of a cytopathic effect of the virus, appear as multinucleated cells with a perinuclear clear haloe. The epithelium is thickened with abnormal keratinisation responsible for the warty appearance of the lesion.

Such flat condylomas when positive for the HPV 16 or 18 serotypes, are high-risk factors for the evolution toward cervical intraepithelial neoplasia (CIN) and carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.

International Patent Application No. WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.

HPV L1 based vaccines are disclosed in WO94/00152, WO94/20137, WO93/02184 and WO94/05792. Such a vaccine can comprise the L1 antigen as a monomer, a capsomer or a virus like particle. Such particles may additionally comprise L2 proteins. Other HPV vaccines are based on the Early proteins, such as E7 or fusion proteins such as L2-E7.

In the vaccine of the invention it is preferred to utilise compositions comprising either an E6 or E7 protein linked to an immunological fusion partner having T cell epitopes.

In a preferred form of the invention, the immunological fusion partner is derived from protein D of Heamophilus influenza B. Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids.

Accordingly, the present invention may employ fusion proteins comprising Protein D - E6 from HPV 16, Protein D - E7 from HPV 16 Protein D - E7 from HPV 18

and Protein D - E6 from HPV 18. The protein D part preferably comprises the first 1/3 of protein D.

The obligate intracellular bacteria *Chlamydia trachomatis* infects mucosal epithelial cells of the conjunctiva and of the urogenital tract, causing a wide spectrum of human diseases such as trachoma and genital infections which can result in long term sequelae. Trachoma, which is endemic in several developing countries, is the world's leading cause of preventable blindness; genital infections, which represent around 3 million cases per year in the US, rend annually 200, 000 women infertile following Chlamydia salpingitis (1). Therefore, this pathogen is a significant public health problem and efforts are made to set up a vaccine against human Chlamydia infections.

Vaccine trials performed in man and non-human primates using the whole organism as immunogen gave serovar-specific protection but some of the vaccinees developed more severe reactions upon reinfection (2). Several studies have demonstrated that the pathology associated with Chlamydia infection is immunologically mediated (3); moreover, a purified Chlamydia 57 kDa (Hsp60) was shown to elicit a pathology similar to reinfection in animals previously infected (4, 5). This observation led to the conclusion that protection against *Chlamydia trachomatis* could only be achieved using a subunit vaccine.

The *Chlamydia trachomatis* species is stereotyped into 15 serovars which are placed into 3 serogroups: the B complex (serovars B, Ba, D, E, L1 and L2), the intermediate complex (serovars F, G, K, L3) and the C complex (serovars A, C, H, I and J) (6). Sexually transmitted diseases (STD) are caused by serovars D to K which cover the 3 serogroups. Thus a subunit vaccine against Chlamydia STD should protect against multiple serovars that are more or less antigenically related.

For the design of a subunit vaccine, much interest has been focused on the serotyping antigen which consist in the 40 kDa major outer membrane protein (MOMP). This protein which was shown to function *in vitro* as a porin (7), is

present during the whole life cycle of the bacteria (8); this principal surface protein is highly immunogenic in humans and animals. The MOMP display 4 variable domains (VD) surrounded by five constant regions that are highly conserved among serovars (9, 10). *In vitro* and *in vivo* neutralizing B-cell epitopes have been mapped on VDs (11, 12, 13, 14, 15) whereas T-cell epitopes have been identified in both variable and constant domains (16, 17). Recombinant MOMP has been expressed in *E. coli* by different authors (18, 19, 20); however, Manning *et al.* shown that their recombinant protein failed to react with a monoclonal antibody that recognize a conformational MOMP epitope (18).

Immunizations with recombinant or purified MOMP followed by homotypic or heterotypic *Chlamydia* challenge have been performed in different animal models with variable effects on the parameters of the infection (21, 22, 23). An elegant experimental model of salpingitis has been developed in mice in which intrauterine inoculation of a human strain of *Chlamydia trachomatis* leads to long term infertility (24, 25). In a heterotypic challenge experiment, Tuffrey *et al.* have shown that parenteral and mucosal immunization with rMOMP absorbed on alhydrogel reduced the severity of the salpingitis and the duration of the lower genital tract colonization, respectively. However, the preparation conferred no protection against infertility resulting from infection (23).

Both cell mediated and humoral immunity seem to play a protective role in the genital pathologies caused by *Chlamydia trachomatis*. However, Rank's group suggests that in mice T-cell mediated immunity is the principal immune mechanism for controlling chlamydial genital disease (26, 27, 28) and CD4 and CD8 positive T-cells have been shown to contribute to anti-chlamydial immunity *in vivo* (29, 30).

In an embodiment of the invention the MOMP antigen is from Serovar 2 and is produced in E.coli by means of recombinant DNA techniques. In such circumstances the protein is produced without its signal sequence.

Antigens derived from or associated with N. gonorrhoea include transferrin binding protein (Tbp). Two proteins are involved in making the Tbp complex - TbpA and TbpB. The gonococcal TbpA DNA/protein sequence is disclosed in WO 92/03467 (University of North Carolina). A recent paper that refers specifically to TbpA and TbpB of gonococcus and how they are required for infection is Mol. Microbiol., 1998 Feb; 27(3): 611-616. Other antigens include the Por B protein , see Proc Natl Acad Sci U S A 1987 Nov;84 (22):8135-8139 and Mol Biol Evol 1995 May;12(3):363-370. Yet a further antigen is a lipopolysaccharide (R type) described in Can J Microbiol 1978 Feb;24(2):117-123. See also J Immunol 1993 Jul 1;151(1):234-243. The FrpB protein is also a candidate antigen; see J Bacteriol 1995 Apr;177(8):2041-2049 and WO 96/31618. A Pilus vaccine is described in J Clin Invest 1981 Oct;68(4):881-888.

Antigens derived from or associated with the pathogen for syphilis include outer membrane proteins of Treponema; see Emerg Infect Dis 1997 Jan;3(1):11-20. A unique physical feature of Treponema pallidum, the venereally transmitted agent of human syphilis, is that its outer membrane contains 100-fold less membrane-spanning protein than the outer membranes of typical gram-negative bacteria, a property that has been related to the chronicity of syphilitic infection. These membrane-spanning T. pallidum rare outer membrane proteins, termed TROMPs, represent potential surface-exposed virulence determinants and targets of host immunity. The outer membrane of T. pallidum been isolated and its constituent proteins identified. Five proteins of molecular mass 17-, 28-, 31-, 45-, and 65-kDa were outer membrane associated. Tromps 1, 2, and 3 were antigenic when tested with serum from infection and immune syphilitic rabbits and humans. A further candidate is outer envelope protein P6; see J Exp Med 1986 Oct 1;164(4):1160-1170. See also Microbiol Rev 1993 Sep;57(3):750-779

Chancroid is a sexually transmitted diseased caused by Haemophilus ducreyi. Antigens derived from or associated with Haemophilus ducreyi include a 18,000 MW outer membrane protein described in Infect Immun 1996 Jun;64(6):1950-1955. A novel lipoprotein expressed by Haemophilus ducreyi is described in Infect Immun

1996 Dec;64(12):5047-5052 A hemoglobin-binding outer membrane protein is involved in virulence expression by Haemophilus ducreyi in an animal model. See Infect Immun 1996 May;64(5):1724-1735. Characterization of the hgbA locus encoding a hemoglobin receptor from Haemophilus ducreyi is described in Infect Immun 1995 Jun;63(6):2194-2200. See also J Med Microbiol 1992 Dec;37(6):413-419 for identification of highly conserved and species-specific polypeptides of Haemophilus ducreyi.

Combination vaccines adminstered or prepared according to the present invention will contain an immunoprotective quantity of the antigens and may be prepared and administered by conventional techniques.

Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective or therapeutic response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 2-100 μg, most preferably 4-40 μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

The amount of antigen in each vaccine dose is an amount which induces an immunoprotective or therapeutically effective response without significant adverse side effects in typical female vaccinees.

Generally it is expected that each dose will comprise 1-1000μg of antigen, preferably 2-100μg, most preferably 4-40μg. The TH-1 inducing adjuvant, for example 3-DMPL, will normally be present in a range of 10-200μg, preferably 25-75μg, especially about 50μg per dose.

The amount of carrier may vary and may be selected according to the knowledge of one skilled in the art. If aluminium hydroxide (alum) or aluminium phosphate is used the amount employed will generally be in the range 100-1000μg, for example 250 – 750μg, preferably about 500μg per vaccine dose.

Typical amounts of each component in the vaccine are antigen (20μg), alum (500μg) and an adjuvant, especially a TH-1 inducing adjuvant such as 3-DMPL (50μg).

In one preferred aspect the vaccine for use in the invention comprises gD2t, 3-DMPL (especially small particle 3-DMPL) and aluminium hydroxide (alum).

In one preferred regimen the vaccine may be given at intervals of 0, 1 and 6 months. Other dosing regimens, including booster doses, may also be used. The vaccine may be administered intramuscularly.

The manufacture of a vaccine according to the invention may be accomplished by conventional techniques, such as described in WO 92/16231. The method typically involves mixing one or more antigens derived from or associated with an STD with an adjuvant, especially a TH-1 inducing adjuvant, and optionally a carrier as hereinabove described. The resulting vaccine composition may be used for administration to female subjects according to the method of the invention, especially sexually active women suffering from or at risk of contracting an STD.

Generally the women will be in an age range of 12-70 years, more usually adolescents and women of 60 or less, for example 14-60, typically 18-45 as in the study described below. In one aspect a suitable group of women includes those

suffering from or at risk of contracting genital herpes infection. The method or use of the invention may, for example, be applied in seronegative healthy consorts of subjects with genital herpes disease.

The invention is illustrated, without limitation, by the following examples, showing results when a herpes vaccine was administered to female subjects. Similar results may be obtained with vaccines against other STDs such as HPV and chlamydia antigens and with combination or polyvalent vaccines against more than one STD, especially combination vaccines comprising an antigen associated with Herpes Simplex, more especially HSV-2 gD or immunological fragments thereof such as gD2t as hereinabove described.

EXAMPLE 1 - DESIGN OF STUDY

<u>Vaccine under study</u>	SmithKline Beecham Biologicals Herpes simplex candidate vaccine (gD2t-20μg) with Alum (500μg) and 3-DMPL (50μg).
<u>Title</u>	A double-blind, randomized, placebo-controlled study to evaluate the efficacy of SmithKline Beecham Biologicals' Herpes Simplex candidate vaccine (gD2t) with 3-DMPL to prevent genital herpes disease in healthy consorts of subjects with genital herpes disease.
<u>Indication/study population</u>	Healthy adult volunteers, male and female, aged 18 to 45 years with negative serological markers of Herpes Simplex infection (HSV-1 and -2) and whose consort has clinical genital herpes disease.

Objectives of the study

Primary

To compare with placebo, during the 17 month period starting one month after the second vaccination, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes clinical disease.

Secondary

To compare with placebo, starting one month after the second vaccination, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes infection.

To compare with placebo after the full vaccination course, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes infection.

To compare with placebo, after the full vaccination course, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes infection during a period of extended clinical follow-up.

To compare with placebo, after the full vaccination course, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes disease.

To compare with placebo, after the full vaccination course, the protective efficacy of gD-Alum-3-DMPL vaccine to prevent genital herpes clinical disease during a period of extended clinical follow-up.

To evaluate, starting one month after the second vaccination, the time to occurrence of disease in each group.

To evaluate, starting one month after the second vaccination, the time to occurrence of infection in each group.

To evaluate, in each group, the number of typical and atypical cases of genital herpes disease.

To evaluate the severity of primary disease in both groups.

To evaluate the humoral and cellular immune response (excluding subjects from study centers initiated after July 1st, 1995) of the vaccine.

To determine serological or immunological correlates for protective efficacy (excluding subjects from study centers initiated after July 1st, 1995).

In case of primary disease or infection, to evaluate the number of subsequent recurrences in the two groups.

To evaluate the safety and reactogenicity of SmithKline Beecham Biologicals' herpes simplex candidate vaccine (with 3-DMPL) in healthy HSV seronegative subjects.

To evaluate the number of cases of oro-labial (or non-genital) herpes disease.

To compare with placebo, starting one month after the second vaccination, the protective efficacy of gD-Alum-3-DMPL to prevent suspected genital herpes signs and symptoms associated with either Western Blot seroconversion to non-vaccinal antigens or with the detection of HSV DNA in a genital swab by PCR.

To evaluate the incidence of genital herpes disease and HSV infection in vaccine recipients during the period of extended clinical follow-up.

<u>Study design</u>

Double-blind, randomized, placebo-controlled study.

Vaccination schedule : 0-1-6 months.

Initial follow-up period - 17 months for each subject starting 1 month after the second vaccination.

Extended follow-up period - 24 months for each subject (from the month 19 visit to the month 43 visit)

Phase A (double blind, vaccine and placebo recipients) - ends when the last subject enrolled completes the initial follow-up period (around the time that the study is unblinded for analysis).

Phase B (open, vaccine recipients only) - begins when the last subject enrolled completes the initial follow-up period (month 19 visit) and ends when the last subject enrolled completes the month 43 visit.

Because there may be a period of several months between the date that the last subject enrolled completes the initial follow-up period and the date that the study is fully unblinded for analysis, (due to the time required for encoding and cleaning of all of the study data collated during the initial follow-up period and phase A of the extended follow-up period), the initial part of phase B of the extended follow-up period may include both vaccine and placebo recipients.

2 groups : I. gD2t-Alum-3-DMPL
Alum-3-DMPL as placebo

Schematic of HSV-007 Study Design - Study periods: Vaccination (V) phase; Initial follow-up* (initial f/u*); Extended follow-up phase A; Extended follow-up phase B.

*Note: The modified "initial follow-up period" now includes months 2-19

<u>Number of subjects</u>	800 couples will be enrolled into the study to allow for at least 640 evaluable subjects.
<u>Primary efficacy endpoint</u>	During the 17-month period, starting one month after the second vaccination (months 2-19), the primary efficacy end-point will be as follows:

Prevention of disease:

A comparison between the two groups of the number of subjects with at least one compatible symptom of genital herpes disease AND either a concurrent positive culture OR appearance of antibodies to non-vaccinal antigens by Western Blot within six months and positive local detection of herpes simplex DNA by Polymerase Chain Reaction (PCR).

	Clinical Symptom	Culture	Antibodies to non-vaccinal antigens	PCR
Disease	+	+	+/-	NA
	+	-	+	+

NA : Not Applicable

Secondary efficacy endpoints

1) Prevention of infection:

A comparison will be made (between vaccine and placebo groups), of the number of subjects who develop antibodies to non-vaccinal antigens (seroconversion) and of subjects who develop disease (culture proven).

This endpoint will be evaluated for the following periods:

Initial period of follow-up (months 2-19)

Months 7-19

Phase A of the extended follow-up

Initial period of follow-up (months 2-19) and phase A of the extended follow-up combined.

Months 7-19 and phase A of the extended follow-up combined.

The analysis of data from phase A of the extended follow-up period will include all events occurring after each subject's month 19 visit and the end of phase A (when the last subject enrolled completes the month 19 visit).

Case definition

		Clinical Symptom	Culture	Antibodies to non-vaccinal antigens	PCR
Infection	Disease	+	+	+/-	NA
Infection	Disease	+	-	+	+
Infection		+/-	NA	+	NA

NA : Not Applicable

2) Prevention of disease between months 7-19

A comparison with placebo after the full vaccination course (months 7-19) of the number of subjects with at least one compatible symptom of genital herpes disease AND either a concurrent positive culture OR appearance of antibodies to non-vaccinal antigens by Western Blot and positive local detection of herpes simplex DNA by Polymerase Chain Reaction (PCR).

3) Prevention of disease during phase A of the extended follow-up period

During phase A of the extended follow-up period, a comparison will be performed between the two groups of the number of subjects with at least one compatible symptom of genital herpes disease AND either a concurrent positive culture OR appearance of antibodies to non-vaccinal antigens by Western Blot and positive local detection of herpes simplex DNA by Polymerase Chain Reaction (PCR).

In addition, this endpoint will also be evaluated for the new initial follow-up period (months 2-19) and phase A of the extended follow-up period combined and also for months 7-19 and phase A of the extended follow-up combined.

4) To evaluate during the 17 month period starting one month after the second vaccination, in each group, the time to occurrence of genital herpes disease.

5) To evaluate during the 17 month period starting one month after the second vaccination, in each group, the time to occurrence of genital herpes infection.

6) To evaluate in each group the number of cases of typical genital herpes clinical disease and of atypical genital herpes disease.

The case definitions are described in the primary end-point.

7) To evaluate in each group the patient's subjective local and general signs and symptoms of genital HSV disease and their duration.

8) To evaluate the humoral (anti-gD2 antibodies by ELISA and anti-HSV neutralizing antibodies) and cellular (lymphoproliferation, secretion of gamma interferon) response to the vaccine (excluding subjects from study centers initiated after July 1, 1995).

9) If clinical efficacy is demonstrated, serological and immunological markers will be extensively evaluated using the sera and Peripheral Blood Lymphocytes stored when scheduled, in an attempt to determine correlates between protective efficacy and laboratory parameters (excluding subjects from study centers initiated after July 1, 1995).

10) In case of primary disease or infection, to evaluate the number of subsequent recurrences of genital herpes in each group.

11) The local and general reactogenicity and the safety will be evaluated after each vaccination by recording the local and general signs and symptoms after each dose and the adverse experiences during the study course. The haematological and biochemical parameters will be checked at baseline and after the last vaccination.
During the extended follow-up period, all serious adverse experiences reported by vaccine recipients will be recorded.

12) To evaluate the number of clinical cases of non-genital herpes disease, including oro-labial herpes disease, in each group.

13) To compare with placebo, during the 17 month follow-up period, starting one month after the second vaccination, the number of vaccine recipients who develop genital herpes signs and symptoms associated with either seroconversion to non-vaccinal antigens by Western Blot (within a six month period from the onset of genital herpes signs or symptoms) or with the detection of HSV DNA in a genital swab by PCR.

14) During phase B of the extended follow-up period, the number of vaccine recipients who develop antibodies to non-vaccinal antigens (seroconversion) and of subjects who develop disease (culture proven) will be analyzed in relationship to the interval since administration of last vaccination. These data will be used to calculate the attack rate of genital herpes disease and infection.

EXAMPLE 2

The analysis of the primary endpoint is based on comparison of attack rates between the vaccine and placebo groups as described in the RAP. The analysis of the secondary endpoints is based on either comparison of attack rates or comparison of time to occurrence of disease or infection endpoints as described below.

Statistical tests are two-sided and performed using SAS software and an α-level of 0.05. It should be noted that many statistical analyses are reported, but for the secondary endpoints the error rate (α) is not under control. Since no adjustments of the α were performed for the secondary endpoints, the p-values must be interpreted cautiously and as descriptive only.

Populations analysed for Vaccine Efficacy

Efficacy analyses are performed on two subject populations: the intention-to-treat population (ITT) and the according-to-protocol population (ATP). The ATP group is also referred to hereinbelow as the per-protocol (PP) group.

The analysis of the according-to-protocol population is the primary analysis. The definition of the ATP (or PP) population is defined by the study period under consideration:

1) For the period between months 2-19, the ATP population consists of subjects:

- who meet all protocol eligibility criteria
- who have received three doses of vaccine/placebo
- or who have received two doses of vaccine/placebo and for whom the considered event (disease or infection) has occurred prior to the month 6 visit
- for whom the considered event (disease or infection) has not occurred before the start of the month 2-19 period.

2) For the period between months 7-19, the ATP population consists of subjects:

- who meet all protocol eligibility criteria
- who have received three doses of vaccine/placebo
- for whom the considered event (disease or infection) has not occurred before the start of the month 7 - 19 period.

The analysis of the ITT population is considered as the secondary analysis. This analysis includes all subjects who received at least one dose of study vaccine and have at least one on-vaccine assessment.

The purpose of the two analyses is to ensure that protocol violations, subject dropouts and withdrawals are not treatment related and do not lead to any selection bias in the efficacy results.

The populations to be included in the immunogenicity and safety analyses will be fully described in the final study report.

Evaluation periods

The evaluation periods during which analyses are performed include:

- months 2-19 (ATP population)
- months 7-19 (ATP population)
- months 0 - 19 (ITT population)

Selected results are shown below, together with a summary of the overall conclusions of the study.

Table 1a. Adjustment of vaccine effect on the occurrence of genital herpes disease by gender- ITT population

Terms fitted in the model	Deviance	Degrees of freedom	p-value
treatment group	320.5561	845	
treatment group, gender	317.2083	844	0.067
treatment group, gender, and the interaction	312.0443	843	0.023

Table 1b. Adjustment of vaccine effect on the occurrence of genital herpes infection by gender- ITT population

Terms fitted in the model	Deviance	Degrees of freedom	p-value
treatment group	510.8654	845	
treatment group, gender	494.9266	844	<0.001
treatment group, gender, and the interaction	491.5551	843	0.066

Distribution of Genital Herpes Disease and HSV Infection Cases by Treatment Group

Table 2a. Genital Herpes Disease Cases by Treatment Group- Males and Females

Interval	Intention-To-Treat (N= 847)		Per-Protocol*	
	Vaccine (425)	Placebo (422)	Vaccine	Placebo
M 0-2	2	7		
M 2-7	9	8		
M 7-19	4	9	3 (349)	7 (349)
M 2-19	13	17	12 (371)	16 (369)
>M19	1	0		
Total	16	24		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Table 2b. Genital Herpes Disease Cases by Treatment Group- Males only

Interval	Intention-To-Treat (N= 579)		Per-Protocol*	
	Vaccine (288)	Placebo (291)	Vaccine	Placebo
M 0-2	2	2		
M 2-7	6	5		
M 7-19	3	3	2 (240)	2 (247)
M 2-19	9	8	8 (252)	8 (261)
>M19	1	0		
Total	12	10		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Table 2c. Genital Herpes Disease Cases by Treatment Group- Females only

Interval	Intention-To-Treat (N=268)		Per-Protocol*	
	Vaccine (137)	Placebo (131)	Vaccine	Placebo
M 0-2	0	5		
M 2-7	3	3		
M 7-19	1	6	1 (109)	5 (102)
M 2-19	4	9	4 (119)	8 (108)
>M19	0	0		
Total	4	14		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Table 3a. Genital Herpes Infection Cases by Treatment Group- Males and Females

Interval	Intention-To-Treat (N=847)		Per-Protocol*	
	Vaccine (425)	Placebo (422)	Vaccine	Placebo
M 0-2	2	10		
M 2-7	17	13		
M 7-19	11	16	10 (349)	16 (349)
M 2-19	28	30	26 (371)	29 (369)
>M19	5	2		
Total	35	41		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Table 3b. Genital Herpes Infection Cases by Treatment Group- Males only

Interval	Intention-To-Treat (N=579)		Per-Protocol *	
	Vaccine (288)	Placebo (291)	Vaccine	Placebo
M 0-2	2	2		
M 2-7	9	8		
M 7-19	6	6	5 (240)	6 (247)
M 2-19	15	14	13 (252)	14 (261)
>M19	3	0		
Total	20	16		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Table 3c. Genital Herpes Infection Cases by Treatment Group- Females only

Interval	Intention-To-Treat (N=268)		Per-Protocol *	
	Vaccine (137)	Placebo (131)	Vaccine	Placebo
M 0-2	0	8		
M 2-7	8	5		
M 7-19	5	10	5 (109)	10 (102)
M 2-19	13	15	13 (119)	15 (108)
>M19	2	2		
Total	15	25		

*For each interval, the number of per-protocol evaluable subjects is shown in the ( )

Preliminary Efficacy Analysis

Primary efficacy endpoint

During the 17-month period, starting one month after the second vaccination (months 2-19), the primary efficacy end-point will be as follows:

Prevention of disease:

A comparison between the two groups of the number of subjects with at least one compatible symptom of genital herpes disease AND either a concurrent positive culture OR appearance of antibodies to non-vaccinal antigens by Western Blot within six months and positive local detection of herpes simplex DNA by Polymerase Chain Reaction (PCR).

Table 4a. Prevention of Genital Herpes Disease- Males and Females

Interval	Population	Efficacy (95% CI)
All	ITT	33.8 (-22.8, 64.3%)
M 2-19	PP	25.4% (-55.5, 64.2%)

Table 4b. Prevention of Genital Herpes Disease- Males only

Interval	Population	Efficacy (95% CI)
All	ITT	-21.2 (-176.2, 46.8%)
M 2-19	PP	3.6% (-171.7, 60.5%)

Table 4c. Prevention of Genital Herpes Disease- Females only

Interval	Population	Efficacy (95% CI)
All	ITT	72.7% (19.1, 90.8%)
M 2-19	PP	54.6% (-46.4, 85.9%)

<u>Secondary efficacy endpoints</u>

1) <u>Prevention of infection</u>:

A comparison will be made (between vaccine and placebo groups), of the number of subjects who develop antibodies to non-vaccinal antigens (seroconversion) and of subjects who develop disease (culture proven) for the initial period of follow-up (months 2-19).

Table 5a. Prevention of Genital Herpes Infection- Males and Females

Interval	Population	Efficacy (95% CI)
All	ITT	15.2% (-30.4, 44.0%)
M 2-19	PP	10.8% (-48.4, 46.4%)

Table 5b. Prevention of Genital Herpes Infection- Males only

Interval	Population	Efficacy (95% CI)
All	ITT	-26.3% (-138.8, 33.2%)
M 2-19	PP	3.8% (-100.5, 53.9%)

Table 5c. Prevention of Genital Herpes Infection- Females only

Interval	Population	Efficacy (95% CI)
All	ITT	42.6% (-3.9, 68.3%)
M 2-19	PP	21.3% (-57.7, 60.8%)

2) Prevention of infection:

A comparison will be made (between vaccine and placebo groups), of the number of subjects who develop antibodies to non-vaccinal antigens (seroconversion) and of subjects who develop disease (culture proven) for months 7-19.

Table 6a. Prevention of Genital Herpes Infection- Males and Females

Interval	Population	Efficacy (95% CI)
M 7-19	PP	37.5% (-35.8, 71.2%)

Table 6b. Prevention of Genital Herpes Infection- Males only

Interval	Population	Efficacy (95% CI)
M 7-19	PP	14.2% (-177.3, 73.5%)

Table 6c. Prevention of Genital Herpes Infection- Females only

Interval	Population	Efficacy (95% CI)
M 7-19	PP	53.2% (-32.2, 83.4%)

3) Prevention of disease between months 7-19

A comparison with placebo after the full vaccination course (months 7-19) of the number of subjects with at least one compatible symptom of genital herpes disease AND either a concurrent positive culture OR appearance of antibodies to non-vaccinal antigens by Western Blot and positive local detection of herpes simplex DNA by Polymerase Chain Reaction (PCR).

Table 7a. Prevention of Genital Herpes Disease- Males and Females

Interval	Population	Efficacy (95% CI)
M 7-19	PP	57.1% (-64.4, 88.8%)

Table 7b. Prevention of Genital Herpes Disease- Males only

Interval	Population	Efficacy (95% CI)
M 7-19	PP	-2.9% (-624.7, 85.4%)

Table 7c. Prevention of Genital Herpes Disease- Females only

Interval	Population	Efficacy (95% CI)
M 7-19	PP	81.3% (-57.5, 97.8%)

To evaluate during the 17 month period starting one month after the second vaccination, in each group, the time to occurrence of genital herpes disease.

Time to occurrence of genital herpes disease for the ITT population is shown in figures 1a (males and females), 1b (males only), and 1c (females only). The efficacy analyses of time to occurrence of genital herpes disease for the ITT population is shown in tables 8a (males and females), 8b (males only), and 8c (females only). Time to occurrence was not analyzed for the

per-protocol populations (months 2-19) since an early difference in survival without disease was observed between vaccine and placebo recipients prior to month 2.

Note: the time to occurrence analysis excludes genital herpes disease cases occurring after month 19.

Table 8a. Prevention of Genital Herpes Disease by Time to Occurrence- Males and Females

Interval	Population	p-value (Log Rank test)	Efficacy (95% CI)
M 0-19	ITT	0.1432	37.96% (-18.27, 67.45%)

Table 8b. Prevention of Genital Herpes Disease by Time to Occurrence- Males only

Interval	Population	p-value (Log Rank test)	Efficacy (95% CI)
M 0-19	ITT	0.8025	-11.54% (-162.55, 52.63%)

Table 8c. Prevention of Genital Herpes Disease by Time to Occurrence- Females only

Interval	Population	p-value (Log Rank test)	Efficacy (95% CI)
M 0-19	ITT	0.013	73.24% (18.69, 91.19%)

Summary and conclusions after detailed analysis of the results of the trial

Demographic characteristics and risk factors evaluation

Overall, of the 847 (425 vaccine and 422 placebo) subjects enrolled, 697 (344 vaccine and 353 placebo) subjects completed the study through to month 19. One hundred and fifty (150) subjects dropped out of the study; none of the drop outs resulted from a serious adverse event.

Three hundred and seventy (370) subjects in the vaccine group and 369 in the placebo group were evaluable in the month 2 - 19 ATP population. Treatment groups were balanced for all demographic characteristics and the selection of protocol compliers for the ATP population analysis did not result in the introduction of bias by treatment groups.

Risk factors that might impact on the rate of acquisition of genital herpes disease or infection were assessed and included duration of relationship prior to study entry, the mean time until separation from the source partner, the frequency of sexual intercourse (at baseline and during the efficacy follow-up period), and the frequency of condom use (at baseline and during the efficacy follow-up period).

These results indicate that the vaccine and placebo groups were balanced at baseline for all risk factors and the balance was maintained during the study. The similarity of the ITT population profile to that of the ATP population in terms of risk factors also confirms that the elimination of the non-compliers to the protocol did not bias the treatment groups.

Sub-analyses by gender indicates that within each gender group, risk factors that might impact on the acquisition of genital herpes disease or infection are balanced by treatment group.

Primary efficacy endpoint analysis

The analysis of the primary efficacy endpoint does not demonstrate vaccine efficacy against genital herpes in a combined population of male and female seronegative healthy consorts of subjects with genital herpes disease.

The results of the primary efficacy endpoint analysis are summarised as follows:

1) The relative vaccine efficacy in the overall population (month 2 - 19 ATP) is 25.4% (95% CI: -55.5, 64.2; $p = 0.449$). The relative vaccine efficacy for the ITT population is 37.9% (95% CI: -16.6, 67.0; $p = 0.143$).

2) A statistically significant gender by group interaction on the efficacy analysis of the ITT population (p=0.03).

3) A separate analysis by gender shows a vaccine efficacy of 54.2 % in the month 2-19 ATP female population (95% CI: -47.7, 85.8; $p = 0.238$) and a statistically significant vaccine efficacy of 72.7% (95% CI: 19.1, 90.8; $p = 0.014$) in the female ITT population. In the male population, there is no evidence of vaccine efficacy. In the month 2-19 ATP male population vaccine efficacy is 3.6% (95% CI: -171, 60.5) and -11.1%, (95% CI; -157.6, 52.1) in the ITT male population.

Several baseline covariates were investigated to determine whether they might influence efficacy outcomes: gender, age, frequency of condom use at baseline, frequency of sexual intercourse and duration of relationships prior to study entry. Tendency towards vaccine efficacy was associated with the female gender, age above 30 years, infrequent condom use, sexual intercourse of less than the median frequency and shorter duration of relationships. These observations applied to both ATP and ITT populations.

Secondary efficacy endpoint analyses

Prevention of genital herpes disease (month 7 - 19)

After 3 doses of the vaccine (between study months 7 - 19), vaccine efficacy of 81.1% (95% CI: -58.9, 97.8) was observed for prevention of genital herpes disease in females (p = 0.111). No tendency toward efficacy was observed in males during the month 7 - 19 observation period (-2.9% vaccine efficacy, 95% CI: -624.7, 85.4; $p = 0.99$). This tendency towards vaccine efficacy in the month 7 - 19 female ATP population is consistent with the observation of vaccine efficacy in females in the ITT analysis of the primary endpoint.

Prevention of HSV infection

A comparison was made between vaccine and placebo groups for the efficacy of the vaccine in preventing HSV infection. Overall, there was no vaccine efficacy against HSV infection. However, consistent with the disease endpoint analyses, a tendency toward vaccine efficacy against HSV infection was suggested in females in the ITT population (vaccine efficacy of 46.0%, 95% CI: -2.1, 71.4; $p = 0.072$) and in the month 7 - 19 ATP population (vaccine efficacy of 52.8%, 95% CI: -33.4, 83.3; $p = 0.184$).

Time to occurrence of genital herpes

Time to occurrence of genital herpes disease was calculated from study entry until the occurrence of the disease. The main analysis has been performed by the logrank test; Kaplan-Meier curves were plotted for each group. In females (month 2 - 19 ATP population), separation of the curves denoting the occurrence of disease cases is apparent from approximately nine months with disease cases continuing to occur in the placebo group. Vaccine efficacy is estimated at 53.6% (95% CI: -54.2, 86.0) in females. In the ITT female population where the separation of the vaccine and placebo curves is apparent from month 0, a statistically significant vaccine efficacy is estimated at 73.2% (95% CI: 18.7, 91.2; p=0.013). No vaccine efficacy is observed for the male population. Again, the results of the "time to occurrence" analysis is consistent with the primary endpoint analysis.

Severity of Genital herpes disease

Parameters including, duration of lesions, duration of symptoms per episode, number of symptoms per episode and intensity of symptom per episode were used to assess severity of disease in both treatment groups. In the combined month 2 - 19 ATP population, duration of symptoms per episode is significantly longer in the small number of cases occurring in the vaccine group ($p = 0.031$). The gender specific severity data also reveals that in females, there is statistically significant higher number of genital herpes disease lesions per episode ($p = 0.010$) in the vaccine group. These observations suggests that while vaccination may prevent mild to moderate disease in the vaccine group, disease with more severe manifestations were not prevented by vaccination.

Overall Conclusions

The analysis demonstrates that while there may be a tendency toward vaccine efficacy against genital herpes, the primary endpoint analysis does not demonstrate vaccine efficacy in a combined population of male and female seronegative healthy consorts of subjects with genital herpes disease. However, a separate sub-analysis by gender group, based on observed gender interaction, **surprisingly shows a tendency towards vaccine efficacy in females** which is statistically significant in the ITT population. There is no evidence of vaccine efficacy in the male population.

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Claims

1. A method of treating a female human subject suffering from or susceptible to one or more sexually transmitted diseases (STDs), which method comprises administering to a female subject in need thereof an effective amount of a vaccine formulation comprising one or more antigens derived from or associated with an STD-causing pathogen and an adjuvant.

2. Use of one or more antigens derived from or associated with an STD-causing pathogen and an adjuvant in the preparation of a vaccine for administration to a human female subject for the prevention and/or treatment of one or more STDs.

3. Method or use according to claim 1 or claim 2 in which the said adjuvant is a TH-1 inducing adjuvant.

4. Method or use according to any one of claims 1 to 3 in which the said one or more antigens includes HSV glycoprotein D or an immunological fragment thereof.

5. Method or use according to claim 4 in which the HSV-2 glycoprotein D is a truncated glycoprotein.

6. Method or use according to claim 5 in which the truncated glycoprotein is HSV gD2 and is devoid of the C-terminal anchor region (gD2t).

7. Method or use according to any preceding claim in which the said one or more antigens includes an antigen derived from or associated with HPV.

8. Method or use according to any preceding claim in which the said one or more antigens includes an antigen derived from or associated with Chlamydia.

9. Method or use according to any preceding claim in which the said one or more antigens includes an antigen derived from or associated with Neiserria gonnorhea.

10. Method or use according to any preceding claim in which the said one or more antigens includes an antigen derived from or associated with Treponema pallidum (syphilis) or Haemophilus ducreyi (chancroid).

11. Method or use according to any preceding claim wherein the antigen or combination of antigens is formulated with a suitable carrier.

12. Method or use according to claim 10 wherein the carrier is aluminium hydroxide (alum), aluminium phosphate or an oil in water emulsion.

13. Method or use according to any preceding claim wherein the adjuvant is the TH-1 inducing adjuvant 3-DMPL.

14. Method or use according to claim 13 in which the particles of 3D-MPL are small enough to be sterile filtered through a 0.22 micron membrane.

15. Method or use according to any one of claims 4 to 14 wherein the vaccine is used to immunise or treat female subjects at risk of contracting herpes simplex infections.

16. Method or use according to claim 15 wherein the vaccine is used to treat or prevent genital herpes infections.

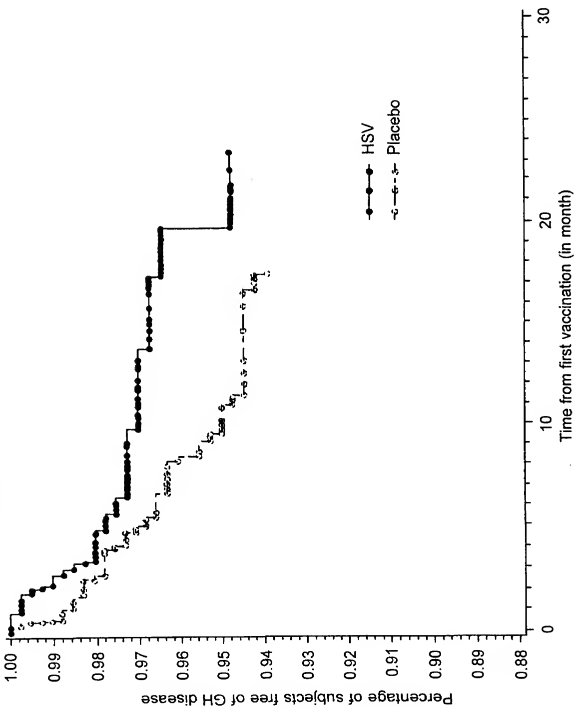
17. Method or use according to claim 15 or 16 in which the vaccine formulation comprises gD2t (1-1000μg), 3-DMPL (10-200μg) and an aluminium salt (100-1000μg).

18. Method or use according to claim 17 in which the vaccine formulation comprises gD2t (20μg), 3-DMPL (50μg) and alum (500μg).

19. Method or use according to any preceding claim wherein the vaccine formulation is administered to, or manufactured for administration to, female subjects at intervals of 0, 1 and 6 months.

20. Method or use according to any preceding claim wherein the vaccine formulation is administered intramuscularly.

Fig. 1a Time to occurrence of genital herpes disease (ITT population; months 0-19)-
Males and females



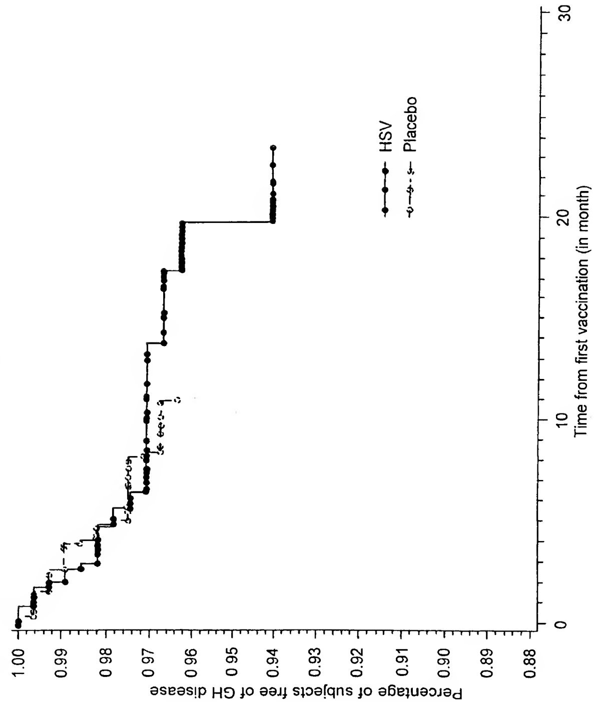
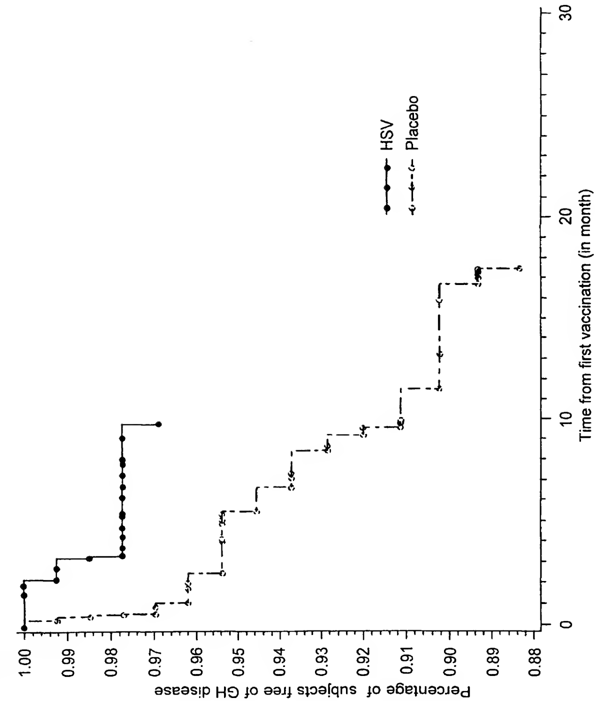


Fig. 1b Time to occurrence of genital herpes disease (ITT population; months 0-19)- Males only

Fig. 1c Time to occurrence of genital herpes disease (ITT population; months 0-19)- Females only



INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 99/06623

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/02 A61K39/245 A61K39/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	STRAUS S.E. ET AL: "Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes." LANCET, (1994) 343/8911 (1460-1463). , XP002128775 the whole document ---	1-12,15, 16,19,20
X	THOELEN S. ET AL: "Safety and immunogenicity of a hepatitis B vaccine formulated with a novel adjuvant system" VACCINE, vol. 16, no. 7, April 1998 (1998-04), pages 708-714, XP002128776 the whole document --- -/--	1-3,7-14

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2000	11/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

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Form PCT/ISA/210 (second sheet) (July 1992)

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	STRAUS S.E. ET AL: "Immunotherapy of recurrent genital herpes with recombinant herpex simplex virus type 2 glycoproteins D and B: results of a placebo-controlled vaccine" THE JOURNAL OF INFECTIOUS DISEASES, vol. 176, 1997, pages 1129-1134, XP000867468 the whole document	1-12,15, 16,19,20
X	WO 92 16231 A (SMITHKLINE BEECHAM BIOLOG) 1 October 1992 (1992-10-01) cited in the application the whole document	1-20
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X	WO 95 17209 A (SMITHKLINE BEECHAM BIOLOG ;MOMIN PATRICIA MARIE (BE); GARCON NATHA) 29 June 1995 (1995-06-29) cited in the application page 4, line 25 -page 5, line 16	1-20
X	"Novel Herpes Vaccine in Development" DOCTOR'S GUIDE TO MEDICAL & OTHER NEWS, 'Online! 10 August 1995 (1995-08-10), page 1-2 XP002128777 Retrieved from the Internet: <URL:http://www.pslgroup.com/dg/5cce.htm> 'retrieved on 2000-01-20! the whole document	1-20
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A	APICELLA M.A. ET AL: "Vaccines for sexually transmitted diseases." CURRENT OPINION IN INFECTIOUS DISEASES, (1996) 9/1 (52-55). , XP000867508 the whole document	1-20

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INTERNATIONAL SEARCH REPORT

. .ernational application No
PCT/EP 99/ 06623

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 [X] Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claimS 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2 [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3 [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2 [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3 [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos

4. [ ] No required additional search fees were timely paid by the applicant Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[...]ional Application No
PCT/EP 99/06623

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